

# Gut Microbiota, Peroxisome Proliferator-Activated Receptors, and Hepatocellular Carcinoma

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**Abstract:** Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. HCC incidence rate is sixth and mortality is fourth worldwide. However, HCC pathogenesis and molecular mechanisms remain unclear. The incidence of HCC is associated with genetic, environmental, and metabolic factors. The role of gut microbiota in the pathogenesis of HCC has attracted researchers' attention because of anatomical and functional interactions between liver and intestine. Studies have demonstrated the involvement of gut microbiota in the development of HCC and chronic liver diseases, such as alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), and liver cirrhosis. Peroxisome proliferator-activated receptors (PPARs) are a group of receptors with diverse biological functions. Natural and synthetic PPAR agonists show potential for treatment of NAFLD, liver fibrosis, and HCC. Recent studies have demonstrated that PPARs take part in gut microbiota inhabitation and adaptation. This manuscript reviews the role of gut microbiota in the development of HCC and precancerous diseases, the role of PPARs in modulation of gut microbiota and HCC, and potential of gut microbiota for HCC diagnosis and treatment.

**Keywords:** gut microbiota, hepatocellular carcinoma, PPARs, carcinogenesis

## Introduction

More than  $1 \times 10^{14}$  microorganisms colonize the human gastrointestinal tract, including bacteria (about  $1 \times 10^4$  bacterial species), archaea, fungi, and viruses.<sup>1</sup> Sequencing results for 1267 human intestinal microbial samples from three continents have shown that human gastrointestinal tract contains more than 9 million genes, which is 150 times the number of all human genes. Among these genes, more than 99% are bacterial, thus intestinal microbiota are also called intestinal microflora.<sup>2</sup> Most of the bacteria in the intestine belong to five phyla: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia. Bacteroidetes and Firmicutes account for 90–95% of all gut microorganisms in healthy people.<sup>1</sup> Gut microbiota play a key role in human health through modulation of metabolism and immunity.<sup>3</sup> Thus, gut microbiota are considered to be the “forgotten organ”.<sup>4</sup> Increasing evidence has demonstrated the involvement of gut microbiota in human diseases, such as inflammatory bowel disease (IBD),<sup>5</sup> type 2 diabetes mellitus (T2DM),<sup>6</sup> obesity,<sup>7</sup> Alzheimer's disease,<sup>8</sup> and heart failure.<sup>9</sup> The underlying mechanisms might be related to microbiota dysbiosis, alteration of bacterial metabolite production, and host immune disorder.<sup>10</sup> Gut microbiota is a dynamic system, which can be influenced by a series of factors, including age,

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immune system formation, geographical location, and short- and long-term dietary structure.<sup>11</sup>

Liver cancer is one of the most common malignant tumors of the digestive system and is characterized by a high mortality rate.<sup>12–14</sup> In 2018, there were 841,080 new liver cancer cases in the world, including 596,574 in males and 244,506 in females. A total of 781,631 patients died of liver cancer in 2018, of which 548,375 were males and 233,256 were females. Liver cancer became the sixth most common cancer in the world and the fourth leading cause of cancer death worldwide.<sup>15</sup> Hepatocellular carcinoma (HCC) makes up 75–85% of primary liver cancer cases. The pathogenesis of HCC is complex and involves a series of factors.<sup>16,17</sup> In China, HCC is mainly attributed to hepatitis B virus (HBV) infection.<sup>18–20</sup> In USA, the predominant HCC etiology is NAFLD.<sup>21,22</sup> Commonly used diagnostic methods for HCC include abdominal ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), selective hepatic angiography, and detection of serum alpha-fetoprotein and  $\alpha$ -L-fucosidase.<sup>23</sup> Liver biopsy is the gold standard for the diagnosis of HCC. However, HCC diagnosis is usually performed with non-invasive techniques, such as dynamic-MRI and/or dynamic-CT. During dynamic-MRI or dynamic-CT detection, comparing with surrounding liver, HCC nodule shows hypersignal intensity in the arterial phase (wash-in), and hypodensity or hyposignal intensity in the venous phase (wash-out). The detection of nodules with wash-in and wash-out in liver cirrhosis patients has an approximately 95% positive predictive value (PPV) for HCC diagnosis.<sup>24</sup> If the patient is non-fibrotic or has no typical HCC imaging manifestations (wash-in and wash-out), then liver biopsy is recommended.<sup>25</sup> However, liver biopsy is not suitable for patients with coagulopathy and hypertension and its sensitivity is not high enough for the diagnosis of early HCC. In addition, there are no early biomarkers and specific symptoms in early stages, resulting in diagnosis at advanced stages for the majority of HCC patients. Hence, a noninvasive diagnostic method for HCC at an early stage is urgently needed. Increasing evidence has indicated the potential of gut microbiota as a novel diagnostic tool for HCC and other precancerous diseases.<sup>26,27</sup>

In the past decade, the findings in experimental and clinical studies demonstrated the role of gut microbiota in the different stages of liver diseases and the development of liver cirrhosis and HCC.<sup>28</sup> The majority of HCC develop in patients with liver cirrhosis.<sup>28</sup> Pathological changes in liver cirrhosis, such as portal hypertension

and decreased gastric acid secretion, directly destroy intestinal barrier and indirectly affect the composition of gut microbiota, promoting the pathological bacteria translocation and the progression of liver diseases.<sup>29</sup> The mechanisms by which gut microbiota promotes the hepatocarcinogenesis involves the leaky gut, gut microbiota dysbiosis, activated lipopolysaccharide (LPS)-Toll-like receptor 4 (TLR4) signaling and the alternation of bacterial metabolites.<sup>30</sup> Modulation of gut microbiome via administration of probiotics or antibiotics might suppress the occurrence and progression of HCC in animal models.<sup>31–33</sup> T-cell checkpoint inhibition is considered the breakthrough in cancer immunotherapy. The anti-programmed cell death-1 (PD1) agent nivolumab has been approved for advanced HCC treatment after sorafenib failure.<sup>34</sup> A recent review indicated that gut microbiota could modulate the efficiency of PD-1 inhibition in melanoma and might influence the efficiency of immune checkpoint therapy in HCC.<sup>28</sup> The present manuscript focuses on the mechanisms by which gut microbiota promotes the development of HCC and the potential of gut microbiome as a novel diagnostic biomarker and therapeutic target for HCC.

## Intestinal Barrier and Gut-Liver Axis

Intestinal barrier prevents harmful substances and pathogens from entering the human body and maintains its stability. A normal intestinal barrier consists of mechanical, biological, immune, and chemical barriers. The mechanical barrier includes the intestinal mucus layer, peristalsis, and epithelium. Normal peristalsis of the small intestine can prevent bacteria from remaining near the intestinal mucosa for too long and reduces the chance of bacteria passing through the mucosa to reach the epithelium. Intestinal flora competes with pathogenic microorganisms for nutrition and forms a biological barrier on the surface of intestinal mucosa, which can prevent the invasion and colonization of pathogenic microorganisms. Some gut bacteria secrete bacteriostatic substances, bacteriocins, and organic acids, which can kill pathogenic bacteria and neutralize toxins.<sup>35</sup> Gut microbiota have been reported to play a crucial role in the maturation of human immune system by regulating maturation and differentiation of T, B, and dendritic cells and maintaining gut homeostasis. In turn, intestinal cells can regulate

intestinal flora through antimicrobial peptides secreted by Paneth cells.<sup>36,37</sup>

The immune barrier consists of intestinal mucosa lymphoid tissue and secretory antibodies of intestinal plasma cells (sIgA).<sup>38</sup> S-IgA produced by the gut-associated lymphoid tissue (GALT) can selectively coat Gram-negative bacteria in the gut, form an antigen-antibody complex, block the combination of bacterial and epithelial cell receptors, stimulate secretion of intestinal mucus, and accelerate the flow of the mucus layer, which can effectively prevent bacterial adhesion to intestinal mucosa.<sup>39</sup> The chemical barrier consists of mucus secreted by the intestinal epithelium, digestive fluid, and bacteriostatic substances produced by resident bacteria.<sup>1,40</sup>

The “gut-liver” axis theory based on a strong anatomical and functional interaction between the liver and the gut was first proposed in 1998.<sup>41</sup> The liver has two independent blood supply sources: the hepatic artery and portal vein. The portal vein makes up approximately 70–75% of liver’s blood supply. The portal vein brings the blood from the spleen and intestines to the liver and contains nutrients absorbed by the digestive tract as well as metabolites and antigens of gut microbiota, such as lipopolysaccharides (LPSs) and bacterial DNA. Bile secreted by the hepatocytes is essential for the digestion and absorption of lipids and fat soluble vitamins. In normal conditions, after specific receptors like nucleotide-binding oligomerization domain-like receptors (NLRs) and Toll-like receptors (TLRs) recognize bacterial metabolites, the liver clears intestinal bacteria and their products, such as LPSs, which are mediators of inflammation and antigens, in order to maintain a stable internal environment.<sup>42</sup> Hence, normal liver function is a part of the intestinal barrier. Of note, constant exposure to low-level bacterial metabolites suppresses the activation of immune cells by TLRs, which is called “endotoxin tolerance,” and activates immune suppression via cytokines, such as transforming growth factor beta (TGFβ), interleukin (IL)-10, and hepatocyte growth factor (HGF).<sup>43</sup> In physiological conditions, gut microbiota can regulate hepatic lipogenesis, bile acid metabolism, oxidation, and levels of inflammation mediators in the liver. In turn, liver regulates gut microbiota through secretion of bile.<sup>44</sup>

In addition, the liver and gut microbiota affect each other in pathological conditions. The clinical studies indicated that patients with poorer liver function have higher gut permeability and mucosal impairment.<sup>45,46</sup> High levels of portal vein LPSs were observed in patients with liver

cirrhosis<sup>46</sup> due to overgrowth of intestinal bacteria and dysbiosis, which are attributed to reduced gastric acid and bile acid secretion and low intestinal motility disturbance caused by liver cirrhosis.<sup>47</sup> The LPSs activate Kupffer cells (KCs) and hepatic stellate cells (HSCs) in the liver, leading to overexpression of inflammatory factors, including tumor necrosis factor (TNF-α) and interleukin (IL)-6, and inflammatory response and oxidative stress of the liver, finally causing hepatocyte DNA damage and the occurrence and accumulation of mutations.<sup>30</sup> In turn, over release of inflammation mediators by KCs and HSCs aggravates intestinal mucosal injury, while portal hypertension results in the edema of intestinal mucosa, which increases its permeability.<sup>48,49</sup> The next section will investigate the effects of gut microbiota on the occurrence and progression of HCC and its underlying mechanisms.

## Mechanism for Intestinal Microorganism Promotion in HCC and Precancerous Diseases

Recent studies have revealed that alteration of the intestinal barrier and composition of gut microbiota, such as LPSs and deoxycholic acid (DCA), promote the development of CLD and HCC by inducing chronic liver inflammation and injury. The peroxisome proliferator-activated receptor (PPAR) pathway has been reported to modulate microbial inhabitation and adaptation, which might influence the onset and progression of HCC.

### Leaky Gut

In pathological conditions, the structure of the gut microbial community is disturbed, leading to the reduction of beneficial microbial organisms, overgrowth of pathobionts or potentially harmful microorganisms, and loss of microbial organisms.<sup>50</sup> This process is called dysbiosis. Gut microbiota dysbiosis, reduced bile acid secretion, overexpression of inflammatory factors in the intestine, and other factors might destroy the intestinal barrier and increase permeability of the intestinal mucosa, leading to translocation of gut bacteria and high levels of bacterial toxins and metabolites in the portal vein, which activates the immune response in the liver. This phenomenon is known as a leaky gut.<sup>51,52</sup> HCC is a consequence of a vicious cycle of chronic liver injury, inflammation, and regeneration and is the terminal stage of CLD, including chronic viral hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), and non-alcoholic

steatohepatitis (NASH).<sup>53</sup> Moreover, the majority of HCCs occur in patients with liver fibrosis and cirrhosis. Hence, CLD, liver fibrosis, and liver cirrhosis are regarded as precancerous HCC diseases.<sup>54</sup> A recent study indicated that the leaky gut and gut microbiota dysbiosis are observed in patients with CLD, and liver cirrhosis and might contribute to the occurrence and progression of HCC in mice.<sup>30</sup> It should be noted that in hepatocarcinogenesis induced by diethylnitrosamine (DEN)+ carbon tetrachloride (CCl<sub>4</sub>), gut sterilization had a strong effect on the inhibition of HCC formation in the late stages and a mild effect in the early stages, demonstrating that tumor-promoting signals induced by the leaky gut mainly occur during late stages of hepatocarcinogenesis.<sup>33</sup>

LPS is a cell wall component of Gram-negative bacteria and is known as an endotoxin. It is released only when the bacteria die. LPS binds to its Toll-like receptor 4 (TLR4) expressed by hepatocytes, KCs, and hepatic stellate cells, promoting hepatic inflammation and subsequent liver fibrogenesis and hepatocarcinogenesis.<sup>33</sup> Dapito et al<sup>33</sup> have found that administration of low dose LPSs through subcutaneous osmotic pumps for 3 months promoted hepatocarcinogenesis induced by DEN plus CCl<sub>4</sub> in mice. The LPS treatment increased the release of inflammatory factors, tumor number, and size. Tumor growth was suppressed after the LPS levels were reduced by the treatment of cocktail antibiotics.<sup>33</sup> When the intestinal barrier is destroyed by a series of factors, the permeability of intestinal mucosa increases and LPSs and other gut microbiota metabolites translocate from the intestine to the portal vein, leading to subsequent liver injury. LPSs can also enter circulating blood, which is called intestinal endotoxemia.<sup>55</sup> Hence, LPS levels in the portal vein and circulating blood to some extent reflect the permeability of intestinal mucosa. High levels of LPSs have been observed in patients with CLD and HCC and in animal models. It has been reported that patients infected with HBV and hepatitis C virus (HCV) have higher LPS serum levels than the uninfected individuals.<sup>56</sup> Ethanol and its metabolite acetaldehyde can destroy the intestinal barrier and increase the LPS levels in the portal vein and serum in rodent models administered with acute and chronic ethanol treatment.<sup>57</sup> Accordingly, plasma LPS concentrations are elevated in patients with ALD.<sup>58</sup> Insulin resistance plays a key role in the pathogenesis of NAFLD. In a high fat diet-induced mouse obesity model, LPS serum levels increased two- to three-fold, inducing insulin resistance by upregulating inflammatory pathways.<sup>59,60</sup> Accordingly, an increase in intestinal permeability was observed in patients

with NAFLD due to alternation of gut epithelial tight junction.<sup>61</sup> More importantly, a study conducted by Lin et al<sup>46</sup> have demonstrated that LPS levels in the portal vein were positively correlated with the severity of liver cirrhosis (Child-Turcotte-Pugh scores) and highest levels were observed in patients with Child-Turcotte-Pugh cirrhosis stage C. Moreover, serum LPS levels were elevated in patients with HCC and HCC animal models.<sup>62,63</sup> In addition, Bellot et al<sup>64</sup> have found that plasma levels of bacterial DNA, which activate Toll-like receptor (TLR)-9, were elevated in patients with CLD. These findings demonstrate that the livers of CLD and HCC patients were exposed to high LPS levels and other bacterial products due to a leaky gut, which might promote the onset and progression of HCC.

## Dysbiosis

The qualitative and quantitative alternations have been observed in gut microbiota of CLD and HCC patients, including a change in bacterial abundance, loss of beneficial bacteria, and increase in pathogens.<sup>65</sup> This process is known as dysbiosis. Dysbiosis can affect the progression of CLD and HCC by altering microbiota metabolites, such as LPSs, short-chain fatty acids (SCFAs), and deoxycholic acid (DCA). By performing 16S rRNA gene sequencing, researchers have attempted to figure out the difference in gut microbiota between CLD or HCC patients and healthy individuals. The relative findings and references have been listed in [Table 1](#).<sup>66–74</sup>

The above findings indicate that gut microbiota dysbiosis in CLD patients is disease-specific. However, the majority of CLD patients experience a stage of liver cirrhosis in the process of developing HCC. Research on gut microbiota dysbiosis in liver cirrhosis patients has included patients with diverse underlying CLD, demonstrating that at least some of the microbiota dysbiosis features in liver cirrhosis are common to different aetiologies, and the features are driven by end-stage liver disease features, including reduced bile secretion, portal hypertension, and changes in intestinal immune barrier.<sup>27,30</sup> Grat et al have reported an abundance of *Escherichia coli* in patients with liver cirrhosis and HCC.<sup>75</sup> Increased levels of *Streptococcus*, *Veillonella*, *Clostridium*, *Prevotella*, and *Enterobacteriaceae*, *Streptococcaceae*, *Veillonellaceae*, *Pasteurellaceae*, and *Fusobacteriaceae* families were observed in patients with liver cirrhosis, as well as decreased levels of *Bacteroides*, *Eubacterium*, and *Alistipes* and *Lachnospiraceae* and *Bacteroidaceae* families<sup>27,76</sup> ([Table 1](#)). In addition, dysbiosis in patients with decompensated liver

**Table 1** Changes in Gut Microbiota for Different Liver Diseases

Liver Disease	Changes in Gut Microbiota	References
ALD	Increased: Genus <i>Bifidobacteria</i> <i>Lactobacilli</i> <i>Proteobacteria</i> <i>Fusobacteria</i> Decreased: Genus <i>Bacteroides</i>	66,67
NAFLD/NASH	Increased: Genus <i>Proteobacteria</i> <i>Fusobacteria</i> <i>Erysipelotrichaceae</i> <i>Enterobacteriaceae</i> <i>Lachnospiraceae</i> <i>Escherichia Shigella</i> <i>Streptococcaceae</i> <i>Blautia</i> Decreased: Genus <i>Prevotella</i>	68,69
CHB	Increased: Genus <i>Megamonas</i> <i>Clostridium sensu stricto</i> <i>Actinomyces</i> <i>Enterobacteriaceae</i> <i>Enterococcus faecalis</i> <i>Faecalibacterium prausnitzii</i> unclassified <i>Lachnospiraceae</i> Decreased: Genus <i>Alistipes</i> <i>Bacteroides</i> <i>Asaccharobacter</i> <i>Butyricimonas</i> <i>Ruminococcus</i> <i>Clostridium cluster IV</i> <i>Parabacteroides</i> <i>Escherichia/Shigella</i> <i>Bifidobacteria</i> Lactic acid bacteria	71,72
CHC	Increased: Genus <i>Streptococcus</i> <i>Lactobacillus</i> <i>Bacteroidetes</i> Decreased: Genus <i>Bifidobacterium</i> Order <i>Clostridiales</i>	73,74
Liver cirrhosis	Increased: Genus <i>Streptococcus</i> <i>Veillonella</i> <i>Clostridium</i>	27,76

(Continued)

**Table 1** (Continued).

Liver Disease	Changes in Gut Microbiota	References
	<i>Prevotella</i> Family <i>Enterobacteriaceae</i> <i>Streptococcaceae</i> <i>Veillonellaceae</i> <i>Pasteurellaceae</i> <i>Fusobacteriaceae</i> Decreased: Genus <i>Bacteroides</i> <i>Eubacterium</i> <i>Alistipes</i> Family <i>Lachnospiraceae</i> <i>Bacteroidaceae</i>	
HCC	Increased: Genera-producing lipopolysaccharides <i>Escherichia coli</i> <i>Actinobacteria</i> Decreased: Butyrate-producing bacterial genera <i>Verrucomicrobia</i>	26,75
HCC in NAFLD cirrhosis versus NAFLD cirrhosis without HCC	Increased: <i>Bacteroides</i> <i>Ruminococcaceae</i> Decreased: <i>Bifidobacterium</i>	78

cirrhosis was more obvious than that in patients with compensated liver cirrhosis, demonstrating that it is the cirrhosis stage and not the underlying CLD that drives gut microbiota dysbiosis in liver cirrhosis.<sup>77</sup>

Many HCC patients have been diagnosed at advanced stages due to the lack of effective strategy for early diagnosis. However, recent research on gut microbiota dysbiosis in HCC patients makes early diagnosis possible. A study conducted by Ren et al<sup>26</sup> collected fecal samples from healthy individuals and liver cirrhosis and HCC patients in East, Central, and Northwest China. Then, fecal microbial diversity and composition were identified. Gut microbiota diversity in cirrhosis patients was lower than that in healthy individuals. However, diversity then increased from cirrhosis to early HCC with cirrhosis. Compared to healthy individuals, the levels of butyrate-producing genera were decreased, while genera-producing LPSs was increased in patients with early HCC (Table 1).



Thirty microbial markers were identified using five-fold cross-validation in a random forest model. Most importantly, these markers were further verified in patients from Central and Northwest China, achieving a cross-regional validation. However, this study has several limitations, influencing the interpretation of results to some extent. First, in the discovery cohort, the patients with early HCC had impaired liver function and higher portal hypertension compared with patients with liver cirrhosis. It might be the severity of liver dysfunction rather than existence of HCC that caused the difference in gut microbiota between the two groups. Second, in the validation cohort, the authors only enrolled healthy individuals and patients with HCC. The diagnostic efficacy of these microbial markers should be further investigated by studies conducted on patients with HCC and patients with CLD without HCC.

In a study conducted on NAFLD patients, fecal microbial diversity was found to decrease from healthy individuals to patients with NAFLD. However, no difference in fecal microbial diversity was found between patients with NAFLD-related cirrhosis and HCC and patients with NAFLD-related cirrhosis without HCC.<sup>78</sup> Compared with patients with NAFLD-related cirrhosis without HCC, patients with NAFLD-related cirrhosis and HCC have higher levels of *Bacteroides* and *Ruminococcaceae* and lower levels of *Bifidobacterium*.<sup>78</sup> However, in another study conducted on NASH patients, no difference in fecal microbial composition was found between patients with and without HCC.<sup>79</sup> The different findings in these studies might be due to the differences in techniques to analyze the samples, enrollment of cohorts, ethnics, geographical position and underlying liver diseases.<sup>80</sup> It should be noted that even in studies on mice, the location of facility might influence the composition of gut microbiota. Moreover, a recent study indicated that gut microbiota in laboratory mice was significantly different from that in wild mice.<sup>81</sup>

Hence, in order to compare the findings and generalize conclusions in different studies, sample collection, bacterial lysis, DNA purification sequencing, bioinformatics and statistical analysis should be standardized in the future studies on gut microbiota in HCC patients.<sup>82</sup> The efficacy and stability of gut microbiota as a diagnostic tool for HCC needs to be further validated in populations from different continents. The combination of microbial markers and current diagnostic strategies for HCC might promote the early diagnosis of HCC.<sup>26</sup>

## LPS-TLR4 Pathway

It has been reported that leaky gut leads to high portal and plasma LPS levels in patients and animal models with CLD and HCC. Liver is the first target of gut microbe-associated molecular patterns (MAMPs). Pattern recognition receptors (PRRs), such as TLR4 and NLRs in the liver recognize MAMPs, especially LPSs. TLR4 is expressed in hepatocytes, HSCs, KCs, and endothelial cells. Activation of the LPS-TLR4 pathway leads to an inflammatory response in the liver.<sup>83</sup> A functional study conducted in germ-free, gut-sterilized, or TLR-deficient rodents demonstrated that the LPS-TLR4 pathway plays a significant role in hepatocarcinogenesis. Gabele et al<sup>84</sup> have found that dextran sulfate treatment can lead to high plasma LPS levels due to the leaky gut, promoting liver fibrosis and subsequent hepatocarcinogenesis in mice with NASH. Chronic administration of low dose LPSs can increase the size and number of tumors in DEN+CCl4-induced HCC. Moreover, cancer size and tumor number in germ-free or antibiotics-administered mice were reduced in the same model. In addition, inhibition of TLR4 reduced the tumor size and number but had no effect on tumor incidence.<sup>33</sup>

Cancer-promoting effect of the LPS/TLR4 pathway is attributed to multiple mechanisms. TLR4 in HSCs is activated after recognizing LPSs, resulting in nuclear factor kappa-B (NF- $\kappa$ B)-mediated overexpression of hepatomito-gen epiregulin, which promotes mitosis. Furthermore, HSCs activated by LPSs secrete extracellular matrix, especially collagen.<sup>85</sup> HSC activation and excessive collagen deposition play essential roles in pathogenesis of liver fibrosis and subsequent liver cirrhosis.<sup>86</sup> In addition, epiregulin-deficiency suppresses hepatocarcinogenesis in mice administered with DEN and CCl4.<sup>33</sup> In addition, activated HSCs secrete vascular endothelial growth factor (VEGF). VEGF promotes angiogenesis, which plays a key role in hepatocarcinogenesis.<sup>85,87</sup>

Activated LPS/TLR4 can suppress hepatocyte apoptosis via the NF- $\kappa$ B-mediated mechanism. Cleaved caspase-3 is a biomarker that promotes cell apoptosis. Cleaved caspase-3 levels were negatively correlated with tumor size in TLR4-deficient and gut-sterilized mice.<sup>33</sup> In addition, activation of the LPS/TLR4 pathway in KCs resulted in overexpression of inflammatory factors, including IL-6 and TNF- $\alpha$ , leading to TNF- $\alpha$ - and IL-6-dependent compensatory hepatocyte proliferation and reduction in hepatocyte apoptosis.<sup>88</sup>

Recent studies have demonstrated that the LPS-TLR4 pathway is related to metastasis and poor prognosis in liver cancer patients.<sup>89</sup> Jing et al have found that activation of the LPS-TLR4 pathway in HCC cells can enhance tumor cell invasive potential and induce NF- $\kappa$ B-mediated epithelial–mesenchymal transition, which is essential for tumor metastasis. In addition, the LPS/TLR4 and AKT +MAPK pathways collaborate to regulate cell proliferation, nitric oxide synthase (NOS) expression, and chemoresistance of HepG2 cells.<sup>90</sup>

## Microbiota Metabolites

In addition to LPSs, it has been reported that other bacterial metabolites, such as DCA and SCFA, regulate HCC progression. Bile acids (BA) are synthesized by hepatocytes and discharged into the intestine through the common bile duct. The intestinal microorganisms metabolize conjugated bile acids into unconjugated primary bile acids and further metabolize them into secondary bile acids in the colon. On the one hand, bile acids exert direct antimicrobial effects by damaging the member of bacterium. On the other hand, bile acids exert indirect antimicrobial effects by increasing farnesoid X-activated receptor (FXR)-induced intestinal antimicrobial peptide synthesis.<sup>91,92</sup> Bile acids regulate the growth and adhesion of gut microbiota, playing a key role in gut microbiota homeostasis.<sup>93</sup> About 95% of the bile acids are absorbed by the intestinal epithelium and then enter the liver through the portal vein. They are then metabolized by the hepatocytes and secreted into the bile to complete the enterohepatic circulation of bile acids. The enterohepatic circulation of bile acids connects liver, intestine, and microbiota together.

Nuclear receptor FXR is the major BA-sensing receptor. BA modulates the proliferation of intestinal epithelial cells, maintaining the integrity of epithelial barrier in a FXR-dependent manner.<sup>94</sup> FXR in hepatocytes has anti-inflammatory and liver regeneration-promoting effects.<sup>95,96</sup> Meanwhile, FXR is the master regulator of bile acids. In physiological conditions, FXR modulates the BA synthesis and transport (discussed elsewhere).<sup>97</sup> Cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) is the rate-limiting enzyme of bile acid synthesis. FXR is the transcriptional repressor of CYP7A1.<sup>91</sup> After sensing bile acids, intestinal epithelial FXR is activated. The activated FXR increases the expression of fibroblast growth factor 15/19 (FGF 15/19), leading to the activation of hepatic fibroblast factor receptor4 (FGFR4). The activated FGFR4 suppresses the expression of CYP7A1, inhibiting the bile acids synthesis in the liver.<sup>98</sup> In physiological conditions, FXR-FGF15/19-

FGFR4 pathway plays a key role in BA homeostasis. Whole-body FXR-deficiency in mice promotes spontaneous hepatocarcinogenesis.<sup>99</sup> In FXR-null mice, intestinal selective reactivation of FXR/FGF15 pathway restored BA homeostasis and inhibited spontaneous HCC development.<sup>100</sup> During gut microbiota dysbiosis and inflammation, intestinal FXR is suppressed, leading to the inhibition of FGF19-FGFR4 signaling.<sup>101</sup> The enterohepatic circulation of BA is disrupted, high-level BA in the enterocyte might aggravate the intestinal inflammation.<sup>98</sup> Meanwhile, the suppressed FGF19-FGFR4 pathway increase hepatic BA synthesis by modulating c-Jun N-terminal kinase (JNK)–extracellular-signal-regulated kinase (ERK)–CYP7A1 pathway.<sup>102</sup> Moreover, during the hepatic inflammation, hepatic nuclear factor kappa-B (NF- $\kappa$ B) signaling is activated, suppressing the expression of FXR in the liver. Inactivated FXR increased the hepatic BA synthesis by regulating downstream small heterodimer partner (SHP)–CYP7A1 pathway.<sup>103</sup> Meanwhile, the hepatic BA transporters controlled by FXR are suppressed when FXR is inactivated.<sup>103</sup> As a consequence, hepatic cholestasis and inflammation are aggravated, which might promote hepatocarcinogenesis.<sup>91</sup>

It has been reported that high-level bile acids may produce cytotoxicity by inducing cell necrosis.<sup>104</sup> DCA is a secondary bile acid, which is produced after 7 $\alpha$ -dehydroxylation of primary bile acids by the gut microbiota. In recent years, the role of DCA in the progression of CLD and HCC has been demonstrated. Increased levels of plasma DCA were found in NASH-induced HCC mouse model, which is induced by the administration of high-fat diet and dimethylbenz(a)anthracene (DMBA).<sup>105</sup> Accordingly, increased abundance of Gram-positive bacterial strains, particularly the *Clostridium* genus, which is capable of producing DCA, was observed in mice fed with a high-fat diet. Conversely, no HCC was observed in mice treated with DMBA and a normal diet. In addition, HCC formation was suppressed after endogenous production of DCA was inhibited by administration of vancomycin, while HCC formation was promoted by the treatment with diets containing DCA.<sup>105</sup> Lipoteichoic acid (LPA) is a component of Gram-positive bacterial cell wall and an agonist of Toll-like receptor 2 (TLR2). LPA collaborates with DCA to activate TLR2 in HSCs, leading to upregulation of senescence-associated secretory phenotype (SASP) and cyclooxygenase-2 (Cox-2). Cox-2-mediated prostaglandin-2 can inhibit antitumor immunity through prostaglandin EP4 receptor, thus promoting HCC progression.<sup>106</sup>

In addition, bile acid metabolism can regulate HCC growth by recruiting C-X-C chemokine receptor type 6+ natural killer





*Enterococcus faecalis* isolated from newborn babies can regulate PPAR $\gamma$ 1 phosphorylation, enhancing DNA binding and transcriptional activation of downstream IL-10 gene in colonic cell lines and mouse primary colonic epithelial cells.<sup>124</sup> IL-10 is an anti-inflammatory cytokine that plays a key role in gut homeostasis. In addition to PPAR $\gamma$ , PPAR $\alpha$  activation also promotes IL-10 release in the intestinal epithelium.<sup>125</sup> After binding with IL-10, gut macrophages convert to anti-inflammatory phenotype C-X-3-C motif chemokine receptor macrophages, modulating the immune response to maintain host intestinal barrier and gut microbial homeostasis. Loss of IL-10 receptors induces spontaneous colitis.<sup>126</sup> In addition, intestinal pathogens also modulate intestinal inflammatory response via the PPAR pathway to colonize the gut. Kundu et al<sup>127</sup> have found that *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) decreased the expression of PPAR $\gamma$  in a TLR4-independent manner in mouse intestinal epithelium, leading to overexpression of inflammatory transcription factors NF- $\kappa$ B and AP-1 and downstream TNF- $\alpha$  and IL-6. The intestinal inflammatory response helps *S. Typhimurium* to colonize the host gut and induce colitis.

As PPAR ligands, SCFAs can regulate gut homeostasis via the PPAR pathway. The overgrowth of facultative anaerobic *Enterobacteriaceae* is considered a marker of dysbiosis. Activation of the PPAR $\gamma$  pathway by butyrate activates  $\beta$ -oxidation of colonocytes and inhibits the expression of inducible nitric oxide synthase (NOS) in the colon. Moreover, T-reg cell expansion induced by SCFAs can cooperate with the activated PPAR $\gamma$  pathway to limit the luminal bioavailability of oxygen and suppress the growth of facultative anaerobic bacteria *Escherichia* and *Salmonella*.<sup>128</sup>

Clostridia-related segmented filamentous bacteria release the inflammatory mediator interleukin (IL)-1 $\beta$ . IL-1 $\beta$  activates intestinal T helper 1 and 17 (Th1 and Th17) cells via the PPAR $\alpha$  pathway.<sup>125</sup> Activated Th1 and Th17 cells produce the inflammatory cytokine interleukin (IL)-22. On one hand, IL-22 is essential for maintenance of gut barrier integrity and intestinal epithelial regeneration. On the other hand, IL-22 activates anti-microbial peptide RegIII $\beta$ , RegIII $\gamma$ , and calprotectin expression in epithelial cells, which is essential for intestinal mucosal immunity.<sup>129</sup> Decreased levels of IL-22, RegIII $\beta$ , RegIII $\gamma$ , and calprotectin increased intestinal inflammation susceptibility and gut dysbiosis in PPAR $\alpha$ -deficient mouse, which could be reversed by PPAR $\alpha$  agonist GW7647.

Yu et al<sup>130</sup> have reported increased incidence of HCC in PPAR $\gamma$ -deficient (PPAR $\gamma^{\pm}$ ) mice compared to wild-type

(PPAR $\gamma^{+/+}$ ) mice in a DEN-induced HCC model. A rosiglitazone (a type of TZD) treatment suppressed the incidence of HCC in PPAR $\gamma^{+/+}$  mice, but not in PPAR $\gamma^{\pm}$  mice. Moreover, overexpression of PPAR $\gamma$  induced by adenoviral infection in Hep3B cells inhibited cell proliferation, induced G<sub>2</sub>/M cell cycle arrest, and triggered extrinsic and intrinsic apoptosis.<sup>130</sup> These findings demonstrate that PPAR $\gamma$  acts as an antioncogene in hepatocarcinogenesis. However, the direct effects of PPAR $\gamma$  on gut microbiota in hepatocarcinogenesis have not been investigated.<sup>115</sup>

Although FXR is the master regulator of bile acids, PPAR can also modulate their metabolism. In hepatocyte, PPAR $\alpha$  is a target gene of FXR.<sup>91</sup> Cytochrome P450 enzymes (CYPs), sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs) are responsible for BA detoxification and can be activated by PPAR $\alpha$ .<sup>131</sup> During liver inflammation, hepatic nuclear factor kappa-B (NF- $\kappa$ B) pathway is activated, leading to the inhibition of hepatic FXR.<sup>91</sup> The inhibited FXR suppresses BA detoxification by decreasing the expression of PPAR $\alpha$  and downstream CYPs, SULTs and UGTs.<sup>131</sup> Moreover, suppressed PPAR $\alpha$  pathway inhibited the expression of multidrug resistance protein 2 (MDR2), MDR3, multidrug resistance-associated protein 3 (MRP3) and MRP4. MRP3 and MRP4 regulate BA efflux to general circulation.<sup>132</sup> MDR2 and MDR3 modulate the canicular biliary secretion of phosphatidylcholine.<sup>133</sup> Combined with the cholestasis induced by the inhibition of FXR during liver inflammation as we discussed in Microbiota Metabolites, liver injury caused by high-level BA are aggravated, which might promote the progression of HCC.<sup>91</sup>

Although there have been no studies focusing on the direct effects of PPARs on the onset and progression of HCC by modulating gut microbiota, recent research has demonstrated the protective effects of natural and synthetic PPAR agonists against CLD by reversing leaky gut and gut dysbiosis.

## Targeting Gut Microbiota for HCC Prevention

The above findings suggest that gut microbiota have an essential role in progression of CLD and hepatocarcinogenesis in animal models and patients. Gut microbiota seems to be a promising target for the treatment of pre-cancerous disease and HCC prevention. Studies conducted in animal models have indicated that administration of antibiotics and probiotics can prevent hepatocarcinogenesis induced by NAFLD and chemical toxins. Moreover, it

has been reported that gut microbiota can modulate the curative effect of targeted therapy.<sup>134,135</sup>

## Antibiotics

Antibiotics including norfloxacin and rifaximin have been commonly used in the clinic to prevent encephalopathy and treat enterogenous infections, such as spontaneous peritonitis in patients with advanced liver cirrhosis or HCC. The suppressive effects of antibiotics on hepatocarcinogenesis are attributed to: 1) amelioration of the leaky gut by reducing the number of intestinal bacteria, including pathogens and potential pathogens and suppressing liver inflammation; 2) production reduction of bacterial metabolites by some antibiotics that promote hepatocarcinogenesis.<sup>30</sup> For instance, vancomycin can inhibit DCA production by eliminating Gram-positive bacteria.<sup>105</sup> Oral supplementation with a cocktail of antibiotics containing ampicillin, neomycin, metronidazole, and vancomycin reduced the tumor size and number in HCC mice induced by diethylnitrosamine (DEN)+ carbon tetrachloride (CCl<sub>4</sub>) or dimethylolbutanoic acid (DMBA) + high-fat diet (HFD).<sup>33,105</sup> It should be noted that suppressive effects of antibiotics on carcinogenesis are more effective at advanced stages than the early stages, suggesting the feasibility of antibiotics administration for HCC patients with advanced liver cirrhosis and those at high risk of HCC. However, long-term treatment by broad-spectrum antibiotics might lead to a decrease in probiotics levels and an increase in drug-resistant bacteria in the intestine, promoting gut microbiota dysbiosis. In addition, the side effects of antibiotics, such as vancomycin nephrotoxicity, restrict their long-term administration.

Hence, an antibiotic with mild side effects, high long-term safety, or even life-long administration for HCC prevention is needed. Rifaximin is a potent broad-spectrum antibiotic that cannot be absorbed by the human body and has extremely high intestinal concentrations and mild side effects.<sup>136</sup> Rifaximin is commonly used in the clinic to treat enterocolitis or traveler's diarrhea and prevent encephalopathy in patients with advanced liver disease and HCC. More importantly, no resistance to rifaximin has been observed in patients receiving long-term treatment. In a DEN/CCl<sub>4</sub>-induced HCC mouse model, the rifaximin treatment effectively reduced tumor size and number. In addition, rifaximin effectively ameliorated portal hypertension and reduced the incidence of spontaneous peritonitis in patients with

liver cirrhosis, thus prolonging their survival.<sup>137,138</sup> However, the effects of rifaximin on the development of HCC in patients with advanced liver disease remain unclear and more experimental and clinical studies are needed.

## Probiotics

Probiotics are active microorganisms that are beneficial to the host as they colonize the human body and change the composition of certain types of host flora. They can promote absorption of nutrients and maintain intestinal health by regulating the immune function and intestinal flora balance. Recent studies have confirmed that probiotics can ameliorate CLD in patients and animal models and suppress hepatocarcinogenesis in animal models. Administration of a VSL#3 mixture containing *Streptococcus*, *Bifidobacterium*, and *Lactobacillus* in patients or rodent models can improve insulin resistance, reduce LPS serum levels, depress the total hepatic fatty acid content and liver inflammation, and attenuate liver injury.<sup>139,140</sup> In a clinical study conducted on patients with HBV-induced liver cirrhosis without overt hepatic encephalopathy, 3-month oral administration of probiotics (containing *Clostridium butyricum* and *B. infantis*) improved patient cognition. There was an increased abundance of beneficial *Clostridium butyricum* and *B. infantis* and a decreased abundance of opportunistic pathogens *Enterococcus* and *Enterobacteriaceae*. The intestinal barrier was improved after the probiotics treatment, resulting in a decreased level of venous ammonia and increased cognitive ability in patients with liver cirrhosis.<sup>141</sup> Accordingly, Dhiman et al<sup>142</sup> have found that the VSL#3 treatment in patients with liver cirrhosis reduces the risk of hospitalization for hepatic encephalopathy and decreases the severity of cirrhosis.

The VSL#3 pretreatment ameliorated gut microbiota dysbiosis, suppressed intestinal inflammation, reduced serum LPS levels, and inhibited HCC growth and multiplicity in HCC rats induced by DEN.<sup>143</sup> Degirolamo et al have found that VSL#3 treatment increased fecal BA excretion and hepatic BA synthesis by inhibiting gut-liver FXR/FGF15 pathway in mice.<sup>144</sup> The effect of VSL#3 on FXR/FGF15 pathway in hepatocarcinogenesis should be further investigated. In a subcutaneous mouse tumor model, the administration of a probiotics mixture Prohep containing *Lactobacillus rhamnosus* GG, viable *Escherichia coli* Nissle 1917, and heat-inactivated VSL#3 suppressed tumor growth and reduced tumor size and weight. An increased amount of beneficial *Prevotella* and

*Oscillibacter* was observed in the treatment group, which produced anti-inflammatory cytokine IL-10. Prohep administration suppressed tumor angiogenesis by reducing the Th17 polarization and secretion of IL-17.<sup>145</sup> Aflatoxins are carcinogenic fungal metabolites that can induce HCC. Gratz et al have found that treatment with a probiotic *Lactobacillus rhamnosus* strain GG, which binds aflatoxins, reduced the hepatotoxicity of aflatoxins by increasing their excretion via the fecal route in rats.<sup>32</sup> The above findings demonstrate that the suppressive effects of probiotics on hepatocarcinogenesis are attributed to amelioration of gut microbiota dysbiosis, improvement of intestinal barrier, inhibition of liver inflammation, modulation of host immune system, and reduction of carcinogen toxicity. Probiotics demonstrate good safety for CLD treatment, as all patients with decompensated cirrhosis tolerated the probiotics treatment.<sup>146</sup> However, there are limited studies on the effects of probiotics on HCC patients. In addition, the majority of probiotics cannot colonize the host digestive tract. The probiotics are also used in different combinations in different studies, making it difficult to compare their effectiveness.

## Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT) refers to the process of transplanting functional bacteria from the feces of healthy people into the intestinal tract of patients, thereby reconstructing intestinal microflora with normal structure and function.<sup>147</sup> In third-century China, Ge Hong used stool from healthy people to treat food poisoning and diarrhea.<sup>148</sup> The use of FMT as a treatment for *Clostridium difficile* infection (CDI) has been approved by the US Food and Drug Administration (FDA) in 2013. FMT cure rate for treating recurrent and refractory CDI is nearly 90%, which is 2–3 times that of the standard antibiotics therapy.<sup>149</sup> In recent years, FMT has shown potential for treatment of inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), obesity, and idiopathic thrombocytopenic purpura.<sup>147,150</sup> It has been reported that transplantation of intestinal microbiota from lean donors can increase insulin sensitivity in patients with metabolic syndrome. Increased gut microbiota diversity and abundance of butyrate-producing intestinal microbiota have been observed after the treatment.<sup>151</sup> The effects of FMT on NASH and liver cirrhosis are currently being evaluated in clinical trials.<sup>152</sup> FMT might suppress hepatocarcinogenesis by ameliorating gut microbiota dysbiosis, reducing the release of LPSs and other cytotoxic products,

and suppressing liver inflammation.<sup>148</sup> This hypothesis needs to be verified with more animal experiments and clinical trials. Moreover, it has not been determined whether gut microbiota restoration by FMT is permanent or transient. Most importantly, the safety of FMT has not been demonstrated. The majority of patients with advanced liver disease have a suppressed immune system. Therefore, they might be infected with pathogens, viruses, and fungi through FMT.

## Prokinetics

Portal hypertension is observed in the majority of patients with advanced liver cirrhosis and HCC, leading to hyperemia and edema of the intestinal mucosa, which influences periodic peristalsis of the small intestine.<sup>153</sup> In addition, liver injury can cause gastrointestinal dysfunction in patients with liver cancer, further aggravating the impact on gastric emptying and small intestine motility. Liver dysfunction can also induce sympathetic nerve excitation, inhibit the parasympathetic nerve, and have adverse effects on gastrointestinal motility, absorption, secretion, and other activities. Gut dysmotility caused by the above factors leads to bacterial overgrowth in the intestine and subsequent LPS translocation. The administration of cisapride—a prokinetic can effectively reduce intestinal permeability, improve intestinal transit, and suppress bacterial overgrowth and LPS translocation in animal models and patients with liver cirrhosis.<sup>154,155</sup> Similar protective effects have been observed in nonselective  $\beta$ -adrenergic blockers (such as propranolol), which reduce sympathetic activity.<sup>156,157</sup> Furthermore, cohort study results<sup>158,159</sup> have demonstrated that long-term administration of propranolol reduces the risk of developing HCC in patients with liver cirrhosis. In addition, propranolol has been reported to suppress proliferation and induce apoptosis of HepG2 and HepG2.2.15 liver cancer cells in vitro.<sup>160</sup>

## PPAR Agonists

Sun et al<sup>161</sup> have reported that a water-insoluble polysaccharide (WIP) isolated from the sclerotium of *Poria cocos* improves hyperglycemia, insulin resistance, hyperlipidemia, and liver steatosis in mice with NAFLD by activating the PPAR $\gamma$  pathway. The WIP treatment increased the SCFA-producing *Lachnospiraceae*, *Alloprevotella*, *Parabacteroides*, *Clostridium* IV, *Ruminococcus*, and *Bacteroides* and decreased pro-inflammatory *Megamonas* and *Proteus*. It also maintained intestinal integrity, demonstrated by the decreased LPS plasma levels.

In a study conducted on rats with ethanol-induced liver disease, a selective PPAR $\delta$  agonist MBX-8025 ameliorated liver injury. The MBX-8025 treatment restored bile acid homeostasis and increased hydrogen-producing *Rikenellaceae*. Hydrogen protected cells from oxidative stress. MBX-8025 decreased pathogenic *Enterococcaceae* and *Coriobacteriaceae*. *Enterococcaceae* is related to liver failure and increased LPS serum levels. *Coriobacteriaceae* can impair cholesterol homeostasis. The MBX-8025 treatment also reversed ethanol-induced gut barrier dysfunction.<sup>162</sup>

Synthetic PPAR $\gamma$  agonists TZDs have been reported to have anti-hepatoma effects in vivo and in vitro. Rosiglitazone suppresses BEL-7402 and Huh7 cell proliferation by upregulating phosphatase and tensin homolog (PTEN) and downregulating Cox-2 via activation of the PPAR $\gamma$  pathway.<sup>163</sup> Rosiglitazone inhibits the migration of BEL-7404 cells via PPAR $\gamma$ -mediated upregulation of PTEN and downregulation of phosphorylated Akt and focal adhesion kinase.<sup>164</sup> Troglitazone has been reported to induce HepG2 cell apoptosis in vivo.<sup>165</sup> Moreover, in an orthotopic metastasis mouse model, rosiglitazone was found to suppress lung metastasis in MHCC97L cells.<sup>166</sup> Endogenous PPAR $\gamma$  ligand 15d-PGJ<sub>2</sub> inhibited proliferation and induced apoptosis of LM3, SMMC-7721, and Huh-7 cells via ROS-mediated JNK activation and Akt downregulation.<sup>167</sup> Natural PPAR $\gamma$  agonist avicularin has been reported to induce apoptosis and inhibit migration and invasion of SMMC7721 and Bel7402 via PPAR $\gamma$  activation induced by ERK and AMPK. Furthermore, avicularin can inhibit lung metastasis in Bel7402 cells.<sup>168</sup>

A PPAR $\alpha$  agonist fenofibrate has been reported to suppress expression of CYP7A1 by inhibiting hepatocyte nuclear factor 4 (HNF-4) in vivo.<sup>169</sup> Fenofibrate was found to increase the biliary phosphatidylcholine secretion in rat hepatocytes by activating MDR3.<sup>170</sup> More importantly, fenofibrate treatment improved cholestasis in patients with primary biliary cirrhosis and primary biliary cholangitis.<sup>171,172</sup> The effect of fenofibrate on bile acids metabolism in hepatocarcinogenesis should be further investigated.

Based on the findings discussed above, it can be concluded that PPAR agonists have anti-hepatoma effects and can potentially be used for treating CLD by modulating gut microbiota. However, the effects of PPAR agonists on the endogenous hepatocarcinogenesis driven by the leaky gut and dysbiosis remain unclear and need further investigation.

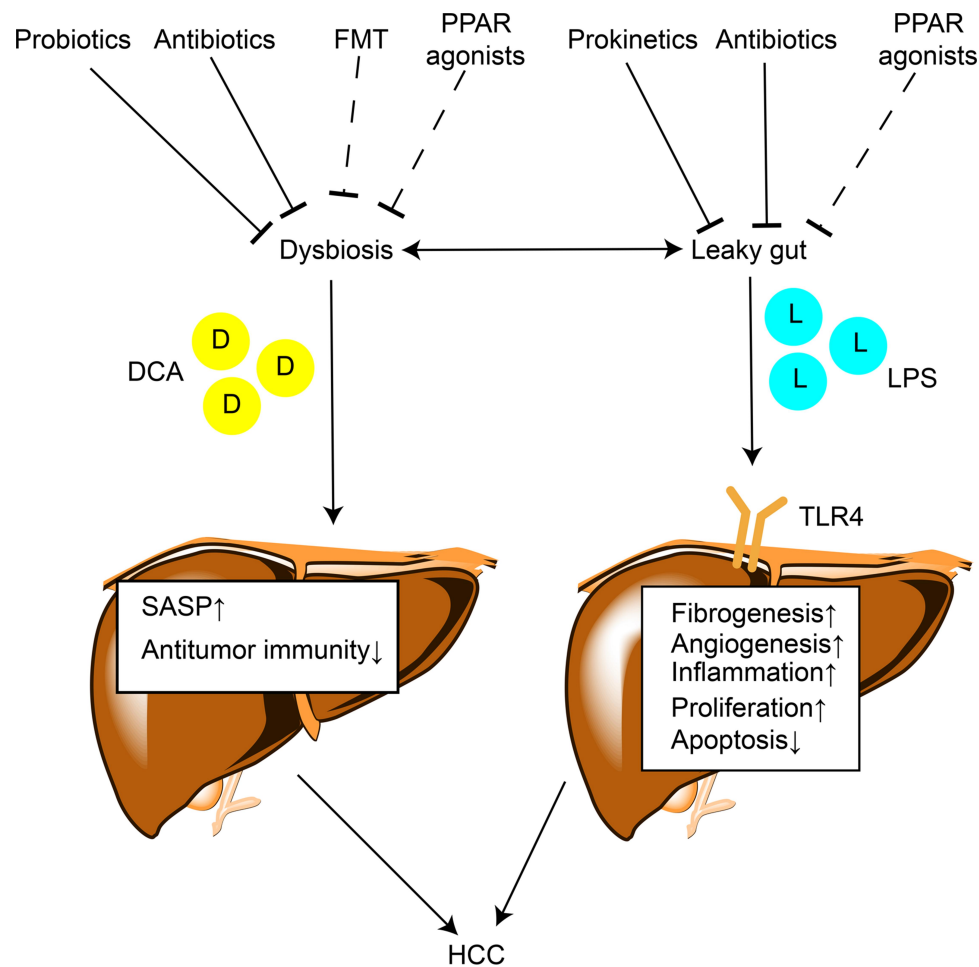
## Modulation of Targeted Therapy

Sorafenib, approved by the FDA in 2007 for the targeted therapy of advanced liver cancer, is a small molecule oral multi kinase inhibitor. Sorafenib has dual antitumor effects. On one hand, it acts on serine/threonine kinase and receptor tyrosine kinase in tumor cells and blood vessels and directly inhibits tumor growth by inhibiting the Raf/MEK/ERK signal transduction pathway.<sup>173,174</sup> On the other hand, it can block the formation of tumor neovascularization by inhibiting VEGF and platelet-derived growth factor (PDGF) receptors and indirectly inhibit tumor cell growth.<sup>175–177</sup> A recent study demonstrated that gut microbiota regulate the VEGF-C secreted by villus macrophages, which is essential for lacteal integrity in mice.<sup>178</sup> More importantly, in a mouse obesity-driven choroidal neovascularization model, gut microbiota dysbiosis induced by high-fat diet destroyed the intestinal barrier and increased the production of VEGF-A, leading to pathological angiogenesis.<sup>179</sup> These findings indicate that gut microbiota might influence the effectiveness of sorafenib by regulating the expression of VEGF, which still needs to be verified via animal experiments and clinical trials.

## Conclusion

A large number of studies have demonstrated the contribution of gut microbiota to the progression of CLD and hepatocarcinogenesis via multiple mechanisms. However, the direct effect of gut microbiota on hepatocarcinogenesis has not been elucidated. The impaired gut barrier and alteration of gut microbiota and their metabolites, such as LPS and DCA, result in chronic liver inflammation and injury, promoting the development of HCC. Studies on dysbiosis in HCC patients suggest the potential of gut microbiota as a noninvasive tool for early diagnosis of HCC. The administration of antibiotics, probiotics, FMT, and prokinetics, which target gut microbiota, might be safe therapeutic options for HCC prevention and treatment. The PPARs can modulate microbial inhabitation and adaptation. PPAR agonists show potential for treating CLD by reversing leaky gut and dysbiosis, indicating the possibility of their use in HCC prevention and treatment (Figure 2). Gut microbiota might modulate efficiency of HCC-targeted therapy. However, animal model findings cannot be directly translated to human patients since CLD and HCC development cannot be perfectly modeled in animals. The





**Figure 2** The mechanisms by which gut microbiota contributes to hepatocarcinogenesis and therapeutic targets. Lines ending in bars represent inhibition and lines ending in arrowheads represent activation. Dotted lines ending in bars mean that the antitumor effect of this strategy needs further validation.

**Abbreviations:** D, deoxycholic acid; L, lipopolysaccharide; SASP, senescence-associated secretory phenotype; TLR4, Toll-like receptor 4; FMT, fecal microbiota transplantation.

clinical trials on patients need to be well designed and administration of antibiotics, probiotics, and proton pump inhibitors before interference should be taken into consideration. Further efforts to determine the roles of gut microbiota during the onset and progression of HCC will assist in finding novel effective and safe strategies for HCC diagnosis, prevention, and treatment.

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## Disclosure

The authors declare no conflicts of interest.

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